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X **PILOT-PLANT PRODUCTION OF CLINICAL-SIZED DEXTRAN
BY ACID HYDROLYSIS OF THE ENZYMATICALLY
SYNTHESIZED HIGH POLYMER**

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**NORTHERN UTILIZATION RESEARCH BRANCH
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PILOT-PLANT PRODUCTION OF CLINICAL-SIZED DEXTRAN
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INTRODUCTION

The methods used in the pilot-plant work here described for the production of dextran by Leuconostoc mesenteroides NRRL B-512 were based on those that were developed in the laboratory by microbiologists, and by physical, organic, and analytical chemists. Initial work in both the laboratory and pilot plant was concerned with the whole-culture method which was quickly developed by an industrial concern. Quite early in the research program, Tsuchiya (10) and Koepsell (3) proposed a process involving the production of a cell-free enzymatic liquor and its use for the conversion of sucrose to dextran. It was apparent that this method would result in a simplified and less expensive procedure for the commercial production of dextran. Consequently, laboratory and pilot-plant work on this process was intensified. On the basis of our early pilot-plant work on the whole-culture method, it was estimated that the production cost of 30 grams, the amount required for 1 unit of unbottled clinical dextran, would be slightly under \$1.00. From the preliminary laboratory work that had been done at that time on the enzymatic method, it appeared that the production cost could be reduced by at least 50 percent by the simple expedient of first producing the enzyme under optimum conditions and then using it for the conversion of sucrose to dextran. The purpose of this bulletin is to describe the pilot-plant methods that have been developed for the production of clinical-sized dextran by a combined enzymatic and hydrolytic process, and to present estimates for the investment and operating costs involved in conducting it on a plant scale.

SUMMARY

A stepwise process has been developed for the production of clinical-sized dextran whereby the fermentation operations have been greatly reduced. Leuconostoc mesenteroides NRRL B-512 is cultured in a suitable medium containing a small amount of sucrose, under conditions such that very little dextran is produced but high concentrations of the dextran-producing enzyme, dextransucrase, are elaborated. The culture liquor is filtered and the filtrate is added to a solution of sucrose in another vessel where the main dextran synthesis takes place. The native dextran is precipitated with

¹ One of the Branches of the Agricultural Research Service, U.S. Department of Agriculture.

methyl alcohol, redissolved, hydrolyzed, and fractionated for the recovery of a clinical-sized fraction meeting current military specifications. Chemical and physical measurements indicate no difference between this product and that produced by the whole-culture method. The yield of the clinical fraction, in the form of a dry, white powder, amounts to approximately 13 percent of the total sucrose used.

Cost estimates are based on a plant with a capacity of 30 million grams of powdered clinical-sized dextran per year. The material would be packaged elsewhere in 500-milliliter bottles, each containing 30 grams of dextran in physiological saline. The capital investment necessary for a plant with a capacity for producing the dextran required for 1 million bottles per year is estimated to be \$655,000. The cost of producing the dextran for one bottle of solution is estimated to be approximately 38 cents. This figure does not include administrative and selling expenses but covers the total estimated manufacturing costs for 30 grams of dry clinical-sized dextran. No estimate has been made for the preparation and packaging of the physiological solution which is a highly specialized and expensive operation. Indeed, the expense of preparing a pyrogen-free solution, of conducting the necessary biological tests for toxicity, and of bottling may well amount to several times the production cost of the dry powder.

PRODUCTION OF DEXTRANSUCRASE

Laboratory results revealed that the yield of the enzyme produced by Leuconostoc mesenteroides NRRL B-512 is influenced profoundly by the pH of the fermenting culture (4, 11). The work was transferred to the pilot plant much earlier than is customary because facilities were available there for automatically controlling the pH of fermentations. In order to determine the optimum conditions for the production of the enzyme, approximately 30 runs of 115 gallons each were conducted. A procedure was developed whereby enzyme potencies of approximately 40 dextransucrase units per milliliter of liquor were obtained with regularity. A dextransucrase unit is defined as that amount of enzyme which converts 1 milligram of sucrose to dextran in 1 hour under specified conditions.

Preparing the inoculum:

Stock cultures of L. mesenteroides NRRL B-512 were maintained on slant medium. After 18-24 hours of incubation at 25° C., the cultures were refrigerated at approximately 4° C. Fresh cultures were prepared once a month. The composition of the slant medium was as follows:

Sucrose.....	2.0%
Yeast extract (Difco)*.....	0.5%
Tryptone (Difco).....	0.25%
Dibasic potassium phosphate..	0.25%
Agar (Difco).....	2.0%
Initial pH.....	7.4 approximately

To initiate the development of an inoculum, a small amount of cells was transferred from the slant culture to 100 milliliters of broth contained in a 300-milliliter Erlenmeyer flask. The flask culture was incubated at 25° C. on a reciprocating shaker for about 16 hours. The composition of the broth was as follows:

Sucrose.....	2.0%
Yeast extract (Difco).....	0.5%
Tryptone (Difco).....	0.25%
Dibasic potassium phosphate..	0.5%
Initial pH.....	7.4 approximately

The inoculum was developed from the broth stage by transferring liquid cultures to liquid medium in successively larger volumes. The rate of inoculation was 5 percent (5 milliliters of culture to 100 milliliters of medium) for every stage except the final one when the volume of inoculum was reduced to 1 or 2 percent. The cultures were incubated at 25° C. for 12 hours under mild aeration, either by shaking the culture flasks or by sparging air into the cultures. The composition of the liquid medium employed to build up the volume of the inoculum was as follows:

Sucrose.....	2.0%
Corn steep liquor (dry basis)	2.0%
Monobasic potassium phosphate	2.0%
R-salts (by volume).....	0.5%

R-salts consist of 40 g. Mg. SO₄ . 7H₂O, 2 g. NaCl, 2 g. FeSO₄ . 7H₂O, 2g. MnSO₄ . H₂O per liter of water solution.

Conducting the Fermentation:

The medium in the fermentor had the following composition:

Sucrose.....	2.0%
Corn steep liquor (dry basis)	2.0%
Monobasic potassium phosphate	0.1%
R-salts (by volume).....	0.5%

* The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

When these ingredients were sterilized, the sucrose was processed separately to prevent the possible formation of products which would inhibit the fermentation. These products apparently are formed when sucrose and corn steep liquor are sterilized together at a pH near 7.0. After adjustment of the pH to 7.5-7.6, the solutions were sterilized continuously at a temperature of 135° C. with a retention time of 3 minutes and then were cooled to 24° C., the fermentation temperature. An inoculum, which was 1 to 2 percent of the total volume fermented, was added. Sterile air at a pressure of 2 to 4 pounds per square-inch gauge was kept above the surface of the liquor in the fermentor and the medium was agitated during fermentation.

As the fermentation proceeded, the pH was allowed to decrease to 6.7. By the addition of dilute sodium hydroxide, the pH was controlled automatically at 6.7 until the fermentation was finished, which usually occurred in approximately 6 hours. The progress of the fermentation could be determined by a decrease in the rate of caustic consumption or by the appearance and subsequent disappearance of the reducing sugar. The enzyme potency also was measured at intervals but because of the time required to perform this analysis, it was not used as a control method. A final determination of the enzyme potency was made on all runs.

Removing the Organisms:

The cells and suspended impurities were filtered from the fermented liquor prior to its use in producing dextran. Although this operation may not be necessary, it appears to be more desirable to remove such materials prior to the formation of dextran rather than after its synthesis. Because the impurities present from the corn steep liquor are more effectively removed at a pH of 7.0, the liquor was adjusted to this pH with dilute caustic just prior to filtration.

A conventional plate and frame filter press was used to conduct the filtration. The filter cloths were made from No. 6 cotton duck and Hyflo Super Cel was used as the filter aid. Numerous experiments were conducted to determine the optimum conditions for filtration and from these it was concluded that the filter cloths should be precoated and filter aid should be added directly to the liquor in order to obtain a satisfactory rate of filtration. It appeared that the optimum procedure for filtering the enzyme liquor was to use 0.1 pound of filter aid per square foot of filtering area as a precoat, and 1 percent filter aid, based on the weight of the liquor, added directly to the slurry to be filtered. This procedure gave a clear filtrate and at least 85 percent of the organisms were removed. The rate of filtration was approximately 8 gallons per hour per square foot of filter area which is quite satisfactory for this operation.

Inasmuch as a pH of 5.0-5.2 had been established by laboratory studies to be optimum for both the stability and the activity of the enzyme (4, 11), the pH of the filtrate was adjusted to this value immediately. Tests showed that the liquor could be stored for at least 30 days at a pH of 5.0-5.2 and at a temperature of approximately 15° C. without appreciable loss of enzyme potency.

Investigations also were conducted on the use of a Sharples Supercentrifuge for removal of the organisms and impurities from the fermented liquor. It was possible to obtain a product comparable in quality to that of the filtered material but the rate of operation was entirely too slow to be commercially feasible.

PRODUCTION AND PURIFICATION OF NATIVE DEXTRAN

The process for producing native dextran by the enzyme method merely involves mixing the required quantities of sucrose solution and enzyme filtrate under prescribed conditions in a suitable vessel. The amount of enzymatic liquor required for the conversion of a given quantity of sugar depends on the potency of the liquor, the temperature, and on the length of time allowed for the reaction to go essentially to completion. The initial rate at which dextran is produced in a reaction mixture is proportional to the enzyme concentration (4). However, when approximately half of the sucrose has been utilized, the rate decreases and finally becomes more nearly proportional to the concentration of the sucrose. Under the conditions used in the pilot plant where it was desired to convert, in a period of 8 hours, essentially all of the sugar contained in a solution with an original concentration of 10 percent, it was found by experiment that approximately 1.75 units of dextranase were required per milligram of sucrose per hour.

The use of a 10-percent solution of sucrose was based on laboratory results which showed that higher initial concentrations produced inordinately high proportions of dextran with molecular weights below the clinical range (5). The 8-hour period for the conversion was chosen quite arbitrarily. Any convenient length of time may be used provided the proper quantity of enzyme is supplied. In the design calculations for the plant, to be described later, a conversion period of 10 hours was selected.

Once the enzymatic liquor has been prepared and its pH adjusted to 5.0, the process becomes quite flexible. The liquor need not be used immediately but may be held at room temperature for several hours with practically no decrease in potency. The time required for the conversion of a given quantity of sucrose to dextran can be altered by varying the amount of enzyme used, and the course of the reaction can be followed by withdrawing samples periodically and measuring the increase in reducing sugar. When the concentration of fructose reaches a maximum, the reaction has gone to completion. Finally, the converted liquor can be held for several hours, with no loss in dextran, before recovery operations are begun.

A run was conducted for the purpose of determining the yield of native dextran and the extent to which it could be purified by redissolving and reprecipitating it a number of times. A 59-gallon batch of enzyme filtrate was produced from 9.8 pounds of sugar and the other necessary ingredients by the method described in the preceding section. The liquor contained 38.5 dextransucrase units per milliliter. It was added, at a pH of 5.0, to 38.2 gallons of distilled water containing 90 pounds of sucrose. Thus, the mixture contained initially 10 percent of sucrose and sufficient enzyme to provide 1.75 dextransucrase units per milligram of sugar to be converted per hour during a period of approximately 8 hours. The total sucrose supplied, however, amounted to 99.8 pounds and all yield calculations were based on this figure.

The reaction mixture had a pH of 5.2 and a temperature of 30° C. It was stirred thoroughly, and 0.1 percent by weight of toluene was added to inhibit growth of contaminating microorganisms. No temperature change occurs during the reaction; hence, in an industrial operation no provisions need be made for cooling or heating the mixture. Agitation is not necessary beyond that required for the original mixing of the enzyme with the sugar. Although the amount of enzyme used was such that the reaction would reach completion in about 8 hours, the mixture, in this instance, was allowed to stand overnight and the dextran was recovered the following day.

Methyl alcohol was added to the reaction mixture for the purpose of precipitating the native dextran. Sufficient alcohol was added to give a final solution containing 50 percent methanol by volume. During the addition of the alcohol the mixture was agitated vigorously to prevent the formation of localized high concentrations of alcohol. The mixture was allowed to stand for 4 hours whereupon the dextran settled to the bottom of the vessel as a thick sirupy liquid, the volume of which amounted to approximately 7 percent of the total volume of the mixture. The supernatant liquor was decanted and retained for recovery of methanol. A small sample of the crude dextran was withdrawn for dextran, ash, nitrogen, and reducing-sugar determinations. The crude dextran was dissolved in distilled water to give a 10-percent solution. Dextran was reprecipitated from this solution in the presence of 50 percent methyl alcohol as before. This procedure was repeated twice, giving 3 reprecipitations of the original crude native dextran.

The yields and purities of the dextran are shown in Table 1, where it will be noted that the first precipitate contains considerable fructose and is high in ash and nitrogen. As the material is subjected to the reprecipitation procedure its purity is improved but it is apparent that no major change results from the second reprecipitation and that the improvement obtained by the third reprecipitation is negligible. From these results, it was concluded that no more than one reprecipitation of native dextran

is required in the purification step prior to its hydrolysis and fractionation for the recovery of a clinical fraction. We have investigated the purity of clinical fractions obtained by processing both the precipitated crude dextran and the material purified by one reprecipitation.

PRODUCTION OF CLINICAL DEXTRAN

Studies were undertaken to determine the yield and quality of clinical material that could be obtained from native dextrans which had undergone only slight purification. The results of previous experiments had established that little change in purification occurred after one reprecipitation. Experiments on the production of clinical material, therefore, were limited to the use of native dextran which had been purified either by one reprecipitation or by only the purification obtained in precipitating it from the reaction liquor.

Processing Native Dextran Purified by One Reprecipitation:

The synthesis solution for the production of native dextran was prepared in the manner described earlier. The original solution consisted of 38.2 gallons of distilled water, 90 pounds of sucrose, and 59 gallons of enzyme filtrate which had a potency of 39 dextransucrase units per milliliter. The sucrose required in the preparation of the enzymatic liquor amounted to 9.8 pounds; hence, the total quantity of sugar used was 99.8 pounds.

After the dextran reaction had gone to completion, a small sample of the liquor was withdrawn for analysis and sufficient methyl alcohol was added to the remainder to give a final solution containing 50 percent methanol by volume. Samples of both the crude native dextran and of the supernatant liquor were taken, and the crude dextran was dissolved in distilled water to give a 10-percent solution. Dextran was reprecipitated from this solution by the use of methanol at a final concentration of 50 percent as before. Samples of the reprecipitated dextran and of the supernatant liquor were removed for analysis.

Since the synthesis solution contained 37.5 pounds of dextran and the yield of reprecipitated material was 33.0 pounds, the recovery amounted to 88.0 percent. The yield of native dextran, based on the total sugar utilized, was 33.1 percent.

Because the yield of native dextran is considerably lower than the theoretical, an attempt was made in the above run to account for the nondextran carbohydrates that are produced during the reaction. The supernatants were analyzed by a quantitative chromatographic method (1, 2, 9) with the results shown in Table 2. In both cases the percentages of the individual constituents total more than 100 percent; but, in view of the nature and complexity of the mixture, the results constitute reasonable material

balances. The synthesis solution and the precipitates also were analyzed and the yields and purities shown in Table 3 were calculated. The analysis for dextran in each of these materials was made by a colorimetric anthrone reaction (8), and fructose was determined by reducing power (6) but corrected for other reducing sugars as determined by chromatographic analysis.

Native dextran from this run was hydrolyzed and fractionated for the production of clinical-sized material by methods that had been developed previously in the laboratory (12). In the initial laboratory studies comparison was made between hydrochloric and sulfuric acids for the hydrolysis, and investigations were carried out on the effect of pH, time, temperature, dextran concentration, and extent of hydrolysis on the yield of clinical-type fraction obtainable. Conditions were developed for the use of either methanol or ethanol as a precipitant for the fractionation of dextran hydrolyzates. The effect of temperature variation during the fractionation process was explored.

Briefly summarized, the laboratory results indicated no major effect on the distribution of hydrolysis products caused by variation of any hydrolysis condition other than extent of conversion, which must be controlled carefully. For control of the extent of hydrolysis, the flow time of the hydrolyzing solution through a capillary-tube viscometer was measured at 25° C. This measurement is expressed as the flow time relative to water, corrected to 5-percent dextran concentration by assuming that $\ln \eta_{rel.}$ is proportional to concentration. As would be expected, the higher molecular-weight fraction decreased in yield and the lower molecular-weight fraction increased as the hydrolysis progressed. The crude clinical fraction was obtained in maximum amount when the hydrolyzing dextran solution was allowed to reach a relative viscosity near 2.65, at which point the reaction was terminated.

The importance of temperature control during fractionation of dextran hydrolyzates was illustrated by the fact that a variation in the temperatures of 5° C. changed the yield of clinical fraction obtained from a given hydrolyzate by as much as 7 to 15 percentage points; e.g., under conditions developed for operation at 25°, the yields of clinical fraction from a hydrolyzate fractionated with methanol at 20°, 25°, and 30° were 21, 36, and 29 percent, respectively, based on the native dextran.

Laboratory preparations of clinical-type fractions were usually carried out on 50 grams of native dextran as the starting material. Translation of these results to the pilot plant, as described in this bulletin, represent, therefore, approximately a three-hundred-fold increase in scale of operations. Comparison of the yields of depolymerized dextran fractions obtained in the hydrolysis and fractionation of once-reprecipitated native dextran indicates a most adequate correspondence between the laboratory results and the yields obtained in the pilot plant as shown in the following table:

Comparison of yields of fractions in laboratory
and pilot-plant operations

	: Yield, percent based on native dextran of		
	: Higher-than-	: Clinical	: Lower-than-
	: clinical-	: fraction	: clinical-
	: molec.-wt.		: molec.-wt.
	: fraction		: fraction
Pilot-plant run	6	38	52
Laboratory fractionation of neutralized pilot-plant hydrolyzate	10	40	50
Laboratory hydrolysis and fractionation of the same native dextran sample	9	37	52

Laboratory experimentation subsequent to the reported pilot-plant runs has established the fact that even higher yields (up to 47 percent) of clinical-type fraction (based on native dextran) can be obtained by suitable adjustment of precipitant limits in the fractionation. The data in the above table lead us to believe that these higher yields would be reproduced easily in larger scale operations. The fact that the pilot-plant yields reported are not necessarily the maximum obtainable should be borne in mind in the interpretation of calculations based on them.

From the 33-pound batch of native dextran produced as described above, only 29.2 pounds were available for processing. The material was dissolved in distilled water to give a solution containing 5.4 percent dextran. A concentration of 5 to 6 percent is recommended. The pH of the solution was adjusted to 1.05 with 6 N H₂SO₄; it was heated to 80° C., and held at this temperature with mild agitation throughout the hydrolysis period. Water was added from time to time to replace that which was vaporized. Viscosity measurements were conducted at regular intervals to determine the progress of the hydrolysis. After approximately 6 hours, the relative viscosity had dropped to 2.66, measured at 25° C., at which time the pH was raised immediately to 7.0 with caustic, and the solution was cooled as rapidly as possible to room temperature. Water was added to the liquor to obtain a dextran concentration of 5 percent, and the hydrolyzed solution was filtered.

Methanol was added slowly to the solution which was being agitated vigorously, until the concentration of alcohol was 42.0 percent by volume. The concentrations of alcohol for fractionation were established for use

at a temperature of 25° C. Although there is a rise in temperature when the alcohol is added, the temperature always was adjusted to 25° C. when the addition of alcohol had been completed. Approximately 1.5 pounds (dry basis) of high-molecular-weight dextran were precipitated under these conditions. The supernatant was decanted and then filtered through a horizontal plate filter. The concentration of alcohol in the filtrate was increased to 49.0 percent. A crude clinical fraction of 16.6 pounds was precipitated at this alcohol concentration.

After the supernatant was decanted, the crude clinical fraction was re-dissolved in water to make a 5-percent solution. Methanol was added to bring the concentration to 41.0 percent by volume, and a precipitate of only 0.1 pound was obtained. After decanting and filtering the supernatant, its alcohol concentration was increased to 49.0 percent, and 12.8 pounds of material were precipitated. A preliminary analysis of this material indicated that its molecular-weight distribution probably would not meet clinical specifications and that another fractionation was necessary.

A 5-percent solution of this precipitate was prepared and fractionations were made at 42.0-percent and 50.0-percent methanol concentrations. From the last precipitation at 50.0 percent methanol, 11.1 pounds of clinical material were recovered after the precipitate had been washed and dehydrated. This is a yield of 38.0 percent based on native dextran and an over-all yield of 12.5 percent based on total sugar used.

Figure 1 is a quantitative flow sheet for the hydrolyzing and fractionating operations. The analytical results on the final product, which are reported in Table 4, indicate that the material meets clinical specifications as required by the Medical Procurement Agency of the Armed Forces, Specifications 1-161-890, May 21, 1952. Other physical and chemical properties of the product showed it to be quite similar to the material produced by the whole-culture method.

Processing Native Dextran Purified Without Reprecipitation:

The procedure for producing clinical material from native dextran which had been precipitated only one time was, in general, similar to that described previously. The quantities of materials used to prepare the native dextran were the same as in the preceding runs. Samples of the synthesis liquor, the precipitated dextran, and the supernatant liquor were analyzed as before. Table 5 gives the composition of carbohydrate material remaining in the supernatant, and the yields and purity shown in Table 6 were calculated from analyses of the synthesis liquor and the precipitated native dextran. The quantity of dextran in the reaction mixture was 34.4 pounds and 31.2 pounds were recovered; hence, the efficiency of the recovery operations was 90.7 percent. The yield of recovered native dextran, based on total sugar, was 31.26 percent.

Thirty pounds of native dextran were hydrolyzed and fractionated. The fractionation procedure was similar to that in the preceding run but with one minor modification. The dextran that was recovered from the second precipitation with 49 percent alcohol by volume was dissolved in water to make a 5-percent solution and this was brought to 50.0 percent alcohol directly, thereby eliminating the removal of any material at a concentration of 42.0 percent alcohol. The precipitate thus obtained was considered to be the final product. Since 11.65 pounds of clinical material were recovered, yields of 38.8 percent based on native dextran or 12.1 percent based on total sugar were obtained. A quantitative flow sheet for the hydrolyzing and fractionating operations for this run is shown in Figure 2.

Investigations were conducted with the clinical dextran from this run to determine the practicability of drying it by the use of a spray dryer. Although the quantity of material was insufficient to permit a thorough study of this problem, satisfactory operating conditions were established which gave an acceptable product.

To prepare the material for drying, the precipitate from the final fractionation was dissolved in water. This solution was heated under vacuum to remove all methanol. It is quite possible that this precautionary measure was unnecessary. Water was added to the solution to obtain a mixture with a dextran concentration of approximately 10 percent. The inlet air temperature to the dryer was approximately 460° F., and the outlet air temperature about 190° F. Under these conditions, a fine, white, powdery product was obtained. This product could be stored readily until the final packaging operations are performed.

Analytical data, reported in Table 7, show that the spray-dried product meets military specifications.

COST ESTIMATES FOR PLANT-SCALE PRODUCTION

Cost estimates have been prepared for a plant producing 30,000 kilograms of dry clinical-sized dextran per year which is sufficient for the preparation of 1 million units annually. A unit is 500 milliliters of a 6-percent solution of dextran in physiological saline. A flow sheet on which cost estimates are based is shown in Figure 3. It is assumed that the plant operates 24 hours per day, 300 days per year. In general the operations for such a plant would be similar to those described previously.

For plant operation, the composition of the medium for producing the enzyme, procedures for preparing the inoculum, and methods for sterilizing and fermenting the medium are the same as those given in the section of this bulletin entitled "Production of Dextranase." Tap water is used in preparing the fermentation medium. In the pilot-plant work here described, distilled water was used in all subsequent operations; but pyrogen-free water will probably be required in commercial practice. The cost

estimates provide for the use of pyrogen-free water at 0.75 cents per gallon. After approximately 6 hours, the fermentation is complete and the enzymatic liquor should contain approximately 40 dextransucrase units per milliliter. For a plant of the above capacity, approximately 950 gallons of enzyme solution must be prepared daily.

The fermented medium is filtered at pH 7.0 to remove suspended matter, and the pH of the filtrate is adjusted to 5.0 to 5.2. Sufficient enzyme filtrate is added to sucrose and pyrogen-free water to obtain a concentration of 10 percent sucrose in the initial solution and to permit the synthesis to be completed in the desired time. Toluene is added to the extent of 0.1 percent. For cost estimation, the time cycle for this operation has been taken as 10 hours with another 2 hours allowed for cleaning the equipment. This cycle can be varied to fit the schedule of the particular plant involved. Temperature of the solution for dextran synthesis is 28° to 30° C.

After synthesis is complete, the dextran is precipitated with methanol at a concentration of 50.0 percent by volume. Approximately 5 hours are required for the precipitate to settle. The supernatant liquor from this precipitation is sent to a methanol recovery still. The precipitate is dissolved in pyrogen-free water to give a 5-percent solution which is then hydrolyzed by the procedure described previously.

The hydrolyzed solution is neutralized, filtered, and then fractionated by the addition of methanol according to the method described earlier. During fractionation, the temperature is controlled automatically at 25° C. Approximately 6 hours are required for each of the various fractions to settle. The supernatant liquors containing the low-molecular-weight fractions are transferred to a methanol recovery still. An over-all loss of methanol of 6 percent has been assumed for the cost calculations. In actual plant operations the loss will surely be less than 6 percent, but during pilot-plant operations the volume of alcohol unaccounted for approached this figure.

After the last fractionation, the clinical material is dissolved in pyrogen-free water to give a 10-percent solution which is then filtered. The solution is spray-dried to give a product, containing about 5 percent moisture, which is ready for final packaging. Although cost estimates were based on the use of a 10-percent solution at this point, it is quite probable that a stronger solution can be fed to the spray drier with a consequent reduction in both the cost of the drier and its operation. The over-all yield of moisture-free clinical material based on total sugar charged to the process has been taken as 13 percent in these calculations. In normal operations this yield should readily be attainable.

Because the product is used for clinical purposes and must be of high purity, all equipment should be constructed of stainless steel. The hydrolyzer should be glass-lined. The precipitation and fractionation tanks must be equipped with agitators which provide for vigorous mixing of the liquors.

Table 8 lists the land, building, and equipment necessary for a plant producing 30,000 kilograms of dextran per year. The total estimated plant investment is \$655,000. Estimated production costs are given in Table 9. The cost of producing 30 grams of clinical material, or one unit, is estimated to be 37.7 cents. Only plant-production costs are included in this figure as administrative and selling expenses have been omitted. Costs for biological tests and for packaging the material in such a manner that it is ready for clinical use have not been included since these operations would probably be performed by firms specializing in the preparation of solutions for injection.

POSSIBLE BYPRODUCTS OR JOINT PRODUCTS

In the above estimates no credit was taken for the fructose that is produced along with the dextran. As will be observed from Table 6, the weight of fructose in the supernatant liquor exceeds that of the native dextran precipitated. The development of a technically and economically feasible process for the recovery of fructose would give a lower net cost for the dextran, or an additional source of profit, or both, depending on the method of cost accounting employed.

Laboratory investigations on the composition of two depolymerized dextran fractions, one above and one below the clinical-sized range, are summarized in Table 10. The fractions were obtained in the pilot-plant hydrolysis of once-reprecipitated native dextran.

As a consequence of the increasing clinical usage of dextran, interest has been expressed in physiological experimentation using dextran fractions different in molecular size from that currently required by military specifications for clinical dextran. A low-molecular-weight dextran fraction has also been proposed as a suitable raw material for sulfation to give a useful blood anticoagulant (7). The data of Table 10 can be used as a guide for estimating the nature and amount of such subfractions that could be obtained as byproducts of the process here described for production of clinical dextran.

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Table 1. Yield and purity of native dextran

Source of dextran	: :	Dry wt. lb.	: :	Yield ^{1/} %	: :	Ash %	: :	Nitrogen %	: :	Fructose ^{2/} %
1st precipitation	:	36.9	:	37.0	:	1.84	:	0.08	:	6.3
1st reprecipitation	:	32.1	:	32.2	:	0.87	:	0.028	:	0.94
2nd reprecipitation	:	32.1	:	32.2	:	0.72	:	0.019	:	0.16
3rd reprecipitation	:	31.7	:	31.8	:	0.67	:	0.019	:	0.10

^{1/} Based on 99.8 lb. of sucrose.

^{2/} By reducing power, assuming no other reducing sugars to be present.

Table 2. Chromatographic composition of carbohydrate in first and second supernatants. (All expressed as glucose equivalent or fructose equivalent, percent of total carbohydrate on control strip.)

Constituent	: : First : supernatant : %	: : Second : supernatant : %
D-Fructose	: : 71.3	: : 76.2
D-Glucose	: : 5.8	: : 5.3
Leucrose	: : 7.9	: : 7.6
Higher Polymers ^{1/} , including levan	: : 12.4	: : 11.6
Indefinite--miscellaneous carbohydrates ^{2/} , of questionable significance	: : <u>8.4</u>	: : <u>3.2</u>
Total	: : 105.8	: : 103.9
Levan (separate determination, expressed as fructose equivalent)	: : 3.6	: : not determined

^{1/} Includes trisaccharide-like material, etc.

^{2/} Maximum possible sucrose is 2 to 3 percent, although sucrose was not proved to be present.

Table 3. Yields of major products and purity of native dextran--one reprecipitation

Material	: Fructose ^{1/} : lb.	: Dextran : lb.	: Ash : %	: Nitrogen : %
Synthesis solution (104 gal.)	: 46.3	37.5		
1st precipitate (39.1 lb.)	: 3.0	33.9	1.62	0.10
1st supernatant	: 43.3	2.8		
Reprecipitate (35.2 lb.)	: 0.65	33.0	0.92	0.035
Supernatant from reprecipitate	: 2.64	0.36		

^{1/} By reducing power, but corrected for other reducing sugars as determined by chromatographic analysis.

Table 4. Properties of clinical dextran produced from the reprecipitated native material

Property	Value
Molecular weight ^{1/}	
Average	71,000
High 6.4%	128,000
Low 9.0%	30,200
Ash, %	0.018
Nitrogen, %	< 0.01
Heavy metals, as Pb, p.p.m.	17
Intrinsic viscosity, deciliter/gram	0.250
pH of 6% aqueous solution	6.25
Buffering capacity (ml. of 0.1 N - NaOH per liter of 6% aqueous solution)	0.1

^{1/} Determined by light scattering.

Table 5. Chromatographic composition of carbohydrate
in supernatant from once-precipitated dextran

Constituent	Composition of carbohydrate, %
Fructose	69.8
Glucose	7.8
Faint or no spot (sucrose area)	1.8
Leucrose	6.8
Isomaltose area	1.4
Tri- or tetrasaccharide area	1.6
Polymers (higher)	10.8
Levan (expressed as fructose)	5.3
Higher glucose polymers (as glucose)	6.6

The last two figures (levan and higher glucose polymers) were obtained from a separate chromatogram spotted much more heavily in order to provide enough levan for the resorcinol method of determination.

Table 6. Yields of major products and purity of native dextran--no reprecipitation

Material	Fructose ^{1/} lb.	Dextran lb.	Ash %	Nitrogen %
Synthesis solution (104 gal.)	48.8	34.4		
Precipitated dextran (35.8 lb.)	2.9	31.2	2.24	0.12
Supernatant	44.2	3.1		

^{1/} By reducing power, but corrected for other reducing sugars as determined by chromatographic analysis.

Table 7. Properties of clinical dextran produced from the once-precipitated native material

Property	Value
Molecular weight ^{1/}	
Average	58,400
High 10.4%	92,000
Low 7.1%	26,500
Ash, %	0.02
Nitrogen, %	<0.01
Heavy metals, as Pb, p.p.m.	40
Intrinsic viscosity, deciliter/gram	0.244
pH of 6% aqueous solution	6.9
Buffering capacity (ml. of 0.1 N - NaOH per liter of 6% aqueous solution)	0.1

^{1/} Determined by light scattering.

Table 8. Estimated investment cost of a plant for producing dextran
annual capacity 30,000 kilograms

Land and Building:

Building (80,000 cu. ft.)	\$ 60,000
Land and Improvements	<u>30,000</u>
	\$ 90,000

Plant Equipment:

<u>Item</u>	<u>Estimated delivered cost</u>
2 - Mixing tanks: 300-gal., with agitator, stainless-steel	\$ 1,500
1 - Inoculum tank: 40-gal., jacketed, with agitator, stainless-steel	1,300
1 - Caustic tank: 60-gal., with agitator, stainless-steel	1,200
1 - Fermentor: 1,200-gal., with agitator, jacketed, stainless-steel	4,900
1 - Synthesis tank: 2,000-gal., with agitator, stainless-steel	5,200
1 - Mixing tank: 200-gal., with agitator, steel	300
1 - Precipitation tank: 4,000-gal., with agitator, jacketed, stainless-steel	8,500
1 - Hydrolysis tank: 1,600-gal., with agitator, jacketed, glass-lined	7,200
3 - Fractionation tanks: 2,700-gal., with agitator, jacketed, stainless-steel	21,000
2 - Methanol storage tanks: 20,000-gal., steel	6,800
1 - Continuous sterilizer: with jet heater, stainless-steel	1,100
1 - Heat exchanger: 56-sq. ft., stainless-steel	1,600
1 - Pump: positive displacement, 10-g.p.m., stainless-steel	2,300

--Continued

Table 8. Estimated investment cost of a plant for producing dextran
annual capacity 30,000 kilograms--continued

<u>Plant Equipment--continued</u> <u>Item</u>	<u>Estimated delivered cost</u>
1 - Filter press: plate and frame, 60-sq. ft. area, stainless-steel	\$ 3,800
1 - Filter press: plate and frame, 90-sq. ft. area, stainless-steel	6,200
1 - Spray dryer: stainless-steel	34,000
1 - Distillation unit: copper, 8 plates, 36-inch diameter	8,000
Pipe and fittings: stainless-steel	10,500
Pumps: for transfer and other uses, stainless-steel	6,600
Instruments and controls	5,000
Laboratory equipment	<u>10,000</u>
Total equipment, delivered	\$147,000
Installation of equipment	42,000
Piping and wiring, installed	113,000
Other construction costs	91,000
Contingencies, engineering and contracting fees	<u>172,000</u>
Total equipment, installed	<u>\$565,000</u>
Total cost of plant, ready for operation	<u>\$655,000</u>

Table 9. Estimated daily operating costs for producing dextran
annual capacity 30,000 kilograms of dextran

Raw Materials and Supplies:

Sucrose	1,690 lb. at \$0.10 per lb.	\$169.00
Corn steep liquor	316 lb. at \$0.04 per lb.	12.64
Miscellaneous chemicals		12.51
Filter aid		7.00
Pyrogen-free water	3,400 gal. at \$0.0075 per gal.	25.50
Methyl alcohol	396 gal. at \$0.335 per gal.	<u>132.00</u>
Total		<u>\$358.65</u>

Utilities:

Electricity	1,270 k.w.h. at \$0.015 per k.w.h.	\$ 19.10
Steam	94,000 lb. at \$0.75 per 1,000 lb.	70.50
Water	25,000 gal. at \$0.075 per 1,000 gal.	<u>1.86</u>
Total		<u>\$ 91.46</u>

Labor and Supervision:

Operators	15 at \$1.75 per hour	\$210.00
Laborers	8 at \$1.50 per hour	96.00
Foremen	3 at \$2.25 per hour	54.00
Chemists	3 at \$2.50 per hour	60.00
Superintendent	1 at \$600 per month	<u>24.00</u>
Total		<u>\$444.00</u>

--Continued

Table 9. Estimated daily operating costs for producing dextran
annual capacity 30,000 kilograms of dextran--continued

Maintenance:

Equipment	5 percent per year on \$565,000	\$ 94.10
Building	2 percent per year on \$ 60,000	<u>4.00</u>
Total		<u>\$ 98.10</u>

Fixed Charges:

Depreciation:

Equipment	10 percent per year on \$565,000	\$188.20
Building	5 percent per year on \$ 60,000	10.00
Taxes and Insurance	3 percent per year on \$655,000	<u>65.50</u>
Total		<u>\$263.70</u>

SUMMARY

	Daily cost	Cost per unit (30 g.)
Raw Materials	\$358.65	\$0.108
Utilities	91.46	0.027
Labor	444.00	0.133
Maintenance	98.10	0.029
Fixed charges	<u>263.70</u>	<u>0.080</u>
Total	<u>\$1,255.91</u>	<u>\$0.377</u>

Table 10. Composition of selected fractions from pilot-plant hydrolysis of once-reprecipitated native dextran

Fraction subfractionated	:MeOH concn. : :(v/v) at which: :fraction pptd.:	:Percent of :total fracn. : :in subfracn. :	:Subfraction properties	
			: DP _N ^{1/}	: η
	: : ---	Entire fraction	264	0.360
	: 40.0	1	---	
Material insol. in	: 41.	21	537	
42 percent MeOH in	: 42.	33	563	
first fractionation	: 43.	13	481	
stage <u>2,3/</u>	: 45.	13	407	
	: 50.	11	315	
	: 55.	6	122	
	: : ---	Entire fraction	13.5	0.100
	: 52.0	9	53	
Material sol. in 49	: 54.	18	63	
percent MeOH in first	: 56.	13	61	
fractionation	: 58.	10	57	
stage <u>2,4/</u>	: 60.	8	45	
	: 62.	7	40	
	: 65.	10	36	
	: 75.	9	25	
	: Supernatant	16	3	

- 1/ Degree of polymerization estimated from reducing power, determined by the procedure of Somogyi.
- 2/ Precipitation at 25° C. from a solution of 5-percent polysaccharide concentration.
- 3/ Fraction deionized before subfractionation.
- 4/ Fraction evaporated in vacuo to sirupy consistency, dehydrated in MeOH, then deionized in aqueous solution prior to subfractionation.

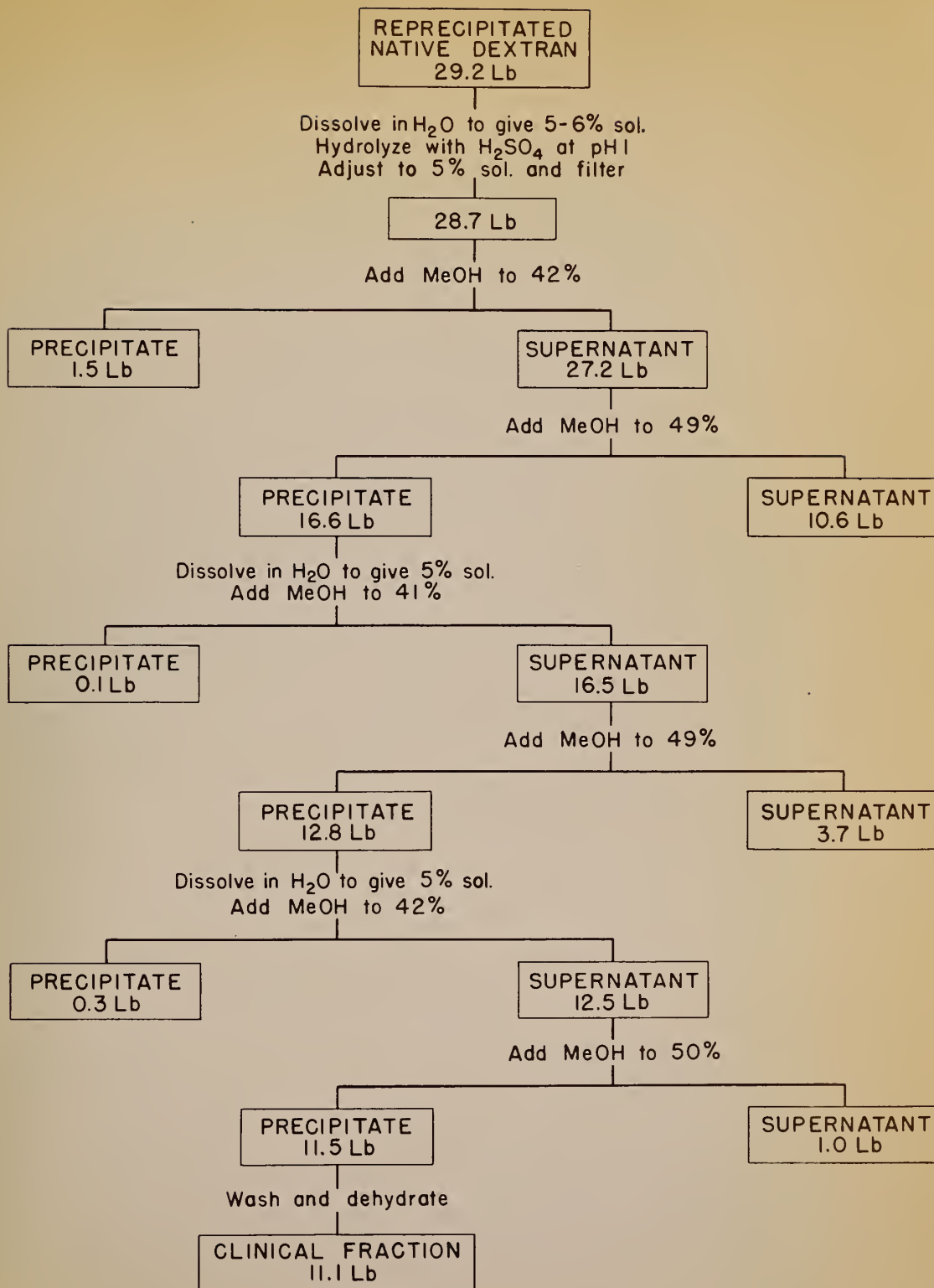


FIGURE 1. Quantitative Flow Sheet for Producing Clinical-sized Dextran from the Reprecipitated Native Product

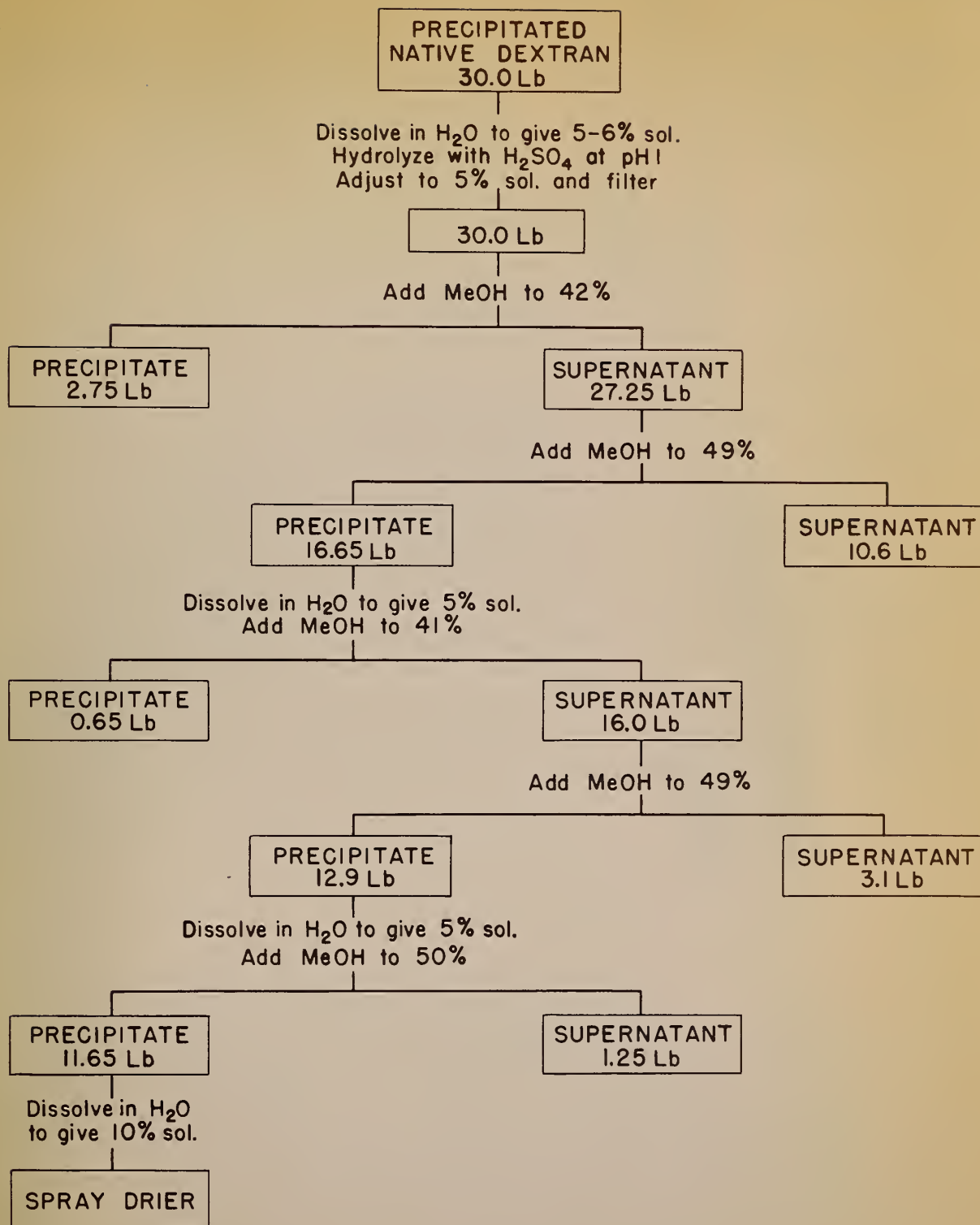


FIGURE 2. Quantitative Flow Sheet for Producing Clinical-sized Dextran from the Once-precipitated Native Product

